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MICROBIOLOGICALLY INFLUENCED CORROSION OF COPPER ALLOYS IN SALINE WATERS CONTAINING SULFATE-REDUCING BACTERIA

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ABSTRACT

Sections of CDA 706 piping and Monel 400 tubing were severely pitted after exposure to marine and estuarine waters. Pits developed under surface deposits of mixed bacterial communities containing 10^4-10^5 sulfate-reducing bacteria (SRB). Localized corrosion was attributed to a combination of differential aeration cells, a large cathode..small anode surface area, concentration of chlorides, development of acidity within the pits and the specific reactions of the base metals with sulfides produced by the SRB. Chlorine and sulfur reacted selectively with the iron and nickel in the alloys. Nickel had been removed from the pitted areas leaving a copper-rich, spongy pit interior. SRB isolated from in-service corroding systems were used to inoculate copper- and nickel-containing foils for laboratory studies. Environmental scanning electron microscopy (ESEM) and energy dispersive x-ray analysis (EDS) were used to characterize the topography and chemical composition of the wet biofilm/corrosion layers. The thickness, tenacity and chemistry of the sulfide layers as well as the severity of localized corrosion varied among the alloys and mixed cultures.

KEYWORDS. Biocorrosion, Cu-Ni seawater piping, sulfate-reducing bacteria, anaerobic corrosion, marine corrosion, estuarine corrosion, CDA 706, Monel 400, ESEM.

INTRODUCTION

Copper and nickel alloys have a long history of successful application in the marine environment. Copper alloys are frequently used for seawater piping systems and heat exchangers due to good corrosion resistance combined with mechanical workability, excellent electrical and thermal conductivity, ease of soldering and brazing, and a resistance to macrofouling. Alloying nickel and iron into copper alters the corrosion product and improves corrosion resistance. CDA 706 (an alloy containing 88.5% copper, 10% nickel and 1.5% iron) has been shown to be the most corrosion resistant copper-based alloy for seawater service.[1]

Nickel alloys such as Monel 400 (containing 66.5% nickel, 31.5% copper and 1.25% iron) have been used extensively in highly aerated, fast-moving seawater environments such as evaporators, heat exchanger pumps and valves, diffusers for steam nozzles in steam ejectors and turbine blades.[2] A passive film similar in structure to that observed on pure nickel is formed on Ni-Cu alloys having more than 30% Ni while alloys containing less than this amount of Ni behave like copper.[3]

Copper and nickel alloys are susceptible to several types of localized corrosion. Premature corrosion failures in predominantly copper alloys have been attributed to erosion corrosion caused by the removal or breakdown of the protective film by mechanical forces, such as local turbulence or impingement[1,4,5] and under-deposit corrosion.[6] In high velocity seawater, nickel alloys are superior to predominantly copper alloys because the protective surface film remains intact under highly turbulent and erosive conditions.[7] Monel is susceptible to pitting and crevice corrosion attack under stagnant conditions.[8-10]

Differential aeration,[11] selective leaching,[12] under-deposit corrosion[13] and cathodic depolarization[14] have been reported as mechanisms for microbiologically influenced corrosion (MIC) of copper and nickel alloys. Pope[15] proposed that the following microbial products accelerate localized attack. CO_2, H_2S, NH_3, organic and inorganic acids, metabolites that act as depolarizers, and sulfur compounds such as mercaptans, sulfides and disulfides. Geesey et al.[16] demonstrated that bacterial exopolymers play a role in the corrosion of copper alloys.

This paper will present case histories of microbiologically influenced corrosion in a CDA 706 seawater piping system and in Monel 400 tubing, and laboratory studies of copper/nickel alloys exposed to anaerobic bacteria including SRB.

MATERIALS AND METHODS

Field Studies

CDA 706. A heavily pitted section of CDA 706 piping (15 cm l x 2.5 cm I. D., wall thickness 3.6 mm) was removed from the seawater piping system of a surface ship after approximately one year of service. During operation of the ship the

seawater piping system had been maintained with continuously flowing seawater (1.6 m/sec) at a maximum temperature of 30°C. However, during the 12-18 months of the shipbuilding process, the system had been operated with estuarine water from the Gulf of Mexico with intermittent flow and long periods of stagnation. At the time of removal, the pitted section was inspected visually, subsectioned and placed in refrigerated 4% glutaraldehyde prepared in filtered seawater. The glutaraldehyde-fixed subsamples were taken through a series of distilled water washes, dehydrated through acetone and xylene washes and air-dried.[17] Surface chemical analyses were performed with a KEVEX 7000 energy dispersive x-ray spectrometer (EDS) coupled to an AMRAY 1000A scanning electron microscope (SEM) . Subsections were sputter-coated with gold for scanning electron micrographs.

Water collected from the seawater piping system was analyzed for salinity[18], dissolved oxygen[18], pH, chlorinity[19], total suspended solids[19], total organic carbon[19] and total dissolved sulfate.[19] Dissolved sulfides were measured using a modification of the method described by Strickland and Parsons[20] that uses a 10 cm cell to increase sensitivity (detection limit is 6.5 ppb) . Numbers of SRB in the flow-through water and in the biofilm were estimated by the most probable number (MPN) method using a medium containing lactate as the electron donor and carbon source and made to the salinity of the seawater.[21]

Monel 400. An experiment was designed to evaluate the corrosion behavior of Monel 400 under conditions of intermittent flow similar to those described for the CDA 706 during shipbuilding. Monel 400 tubes (6.0 meters x 20 mm I.D., wall thickness 1.9 mm) were maintained with intermittently flowing (1.5 m/sec) and with stagnant Gulf of Mexico water for approximately six months. After one month of continuous flow, the water in the tube sections was allowed to stagnate for one week after which the flow was resumed. At the conclusion of the test period, tubes were cross-sectioned, visually inspected, scraped for SRB enumeration and prepared for SEM/EDS analyses as previously described. Gulf of Mexico water was also collected and analyzed for the parameters described previously.

Laboratory Testing

Sampling and Maintenance of Cultures. SRB used in the following experiments were isolated, characterized and maintained at the Naval Surface Warfare Center. The enrichment medium used to isolate and maintain the mixed microbial cultures contained lactate as the electron donor and carbon source for growth.[21] The medium was supplemented with 2.5% (wt/vol) NaCl for enrichment of marine microbes. All cultures were grown anaerobically at room temperature in sealed bottles.

Table 1 shows the location from which the mixed microbial cultures (obligate and facultative anaerobes, SRB and non-sulfate reducers) were originally isolated. Cultures I-V were obtained by aseptically scraping 3" x 3" corrosion coupons in a constant immersion flume tank at NAVSWC/Ft. Lauderdale, FL. Culture VI was obtained from fouling deposits in a sea chest (seawater piping system) of a surface ship at Long Beach Naval Station in Long Beach, CA. Culture VII was isolated from moisture trapped under the cargo ramp of a C-130 transport plane. This aircraft was at the Naval Aviation

Depot (NADEP) at Cherry Point, NC for corrosion maintenance where the ramp area showed signs of blistering and/or peeling of the protective epoxy/polyurethane coatings. Cultures I-VII were used to inoculate 1 cm^2 copper-containing coupons (99Cu, 90Cu:10Ni, 70Cu:30Ni and brass [4Fe:27Zn:69Cu]). The coupon cultures were maintained for 4 to 5 months with the cells fed every 7-10 days.

Characterization of SRB From the Mixed Communities. Postgate medium B[22] was used for the salt tolerance test for the SRB pure cultures. NaCl was added in 0.5% increments (range tested was 0% to 4% NaCl). SRB colony types were tested using the desulfoviridin test.[22] One milliliter of each culture was pelleted in 1.5 ml microfuge tubes and suspended in 100 microliters of growth medium. Cells were lysed by adding 50 microliters of 2N NaOH. The tubes were vortexed and the lysate was immediately viewed under longwave UV irradiation (365 nm). A red fluorescence indicates the presence of the desulfoviridin pigment and is evidence for the presence of *Desulfovibrio*.

Single colonies of each SRB were streaked onto Postgate medium B plates supplemented with the following antibiotics: ampicillin, 50 micrograms/ml, tetracycline, 15 micrograms/ml, kanamycin, 50 micrograms/ml, streptomycin, 25 micrograms/ml, and chloramphenicol, 10 micrograms/ml. Growth within 2 days meant the SRB were resistant to that antibiotic. After 5 days no growth was scored as sensitive to the antibiotic. Ampicillin was purchased from ICN Biochemicals (Cleveland, OH) and the other antibiotics were purchased from USB Corporation (Cleveland, OH).

At the end of the exposure periods, surface topography and chemistry were documented using an Electroscan Model E-30 environmental scanning electron microscope and a Tracor Northern Model 5502 energy dispersive x-ray spectrometer (ESEM/EDS). Coupons were removed from the culture medium, carried through a series of salt water/distilled water washes and examined directly from distilled water.

RESULTS

Field Studies

CDA 706. The surface of the CDA 706 pipe was heavily pitted (Figure 1a). Surface deposits were thick and multilayered, varying in color with depth. Outermost layers were dark green to reddish-brown. Bright bluish-green deposits were found on the surface in unpitted areas. Pit interiors were black and pit depths were typically 1.7-2.0 mm. A cross-section through the pitted areas exposed subsurface bacteria within the thin, black deposit and a spongy subsurface (Figure 1b). Scrapings from the area within the pits indicated the presence of 1×10^5 SRB cm^{-2} compared to 1×10^1 SRB cm^{-2} detected within the surface film on the unpitted areas. The EDS spectrum of the base metal showing the relative concentrations of iron, nickel and copper is shown in Figure 2a. An EDS spectrum taken in the bacterial layer showed concentrations of aluminum, silicon, phosphorus, sulfur, calcium and chlorine in addition to elevated amounts of iron and nickel (Figure 2b,c). The EDS spectrum of the spongy layer under the bacteria indicated an accumulation of phosphorus and a depletion of nickel (Figure 2d).

Water removed from the failed pipe section was typical of seawater (salinity, 35 °/₀₀, dissolved oxygen, 6.4 mg l^{-1}, pH, 8.2; total organic carbon, 1.8 mg l^{-1}, total suspended solids, 3.0 mg l^{-1}, and total dissolved sulfate, 2.4 g l^{-1}). Dissolved sulfides were below detection limits. The SRB concentration in the bulk seawater was typically 1×10^1-10^2 cc^{-1}.

Monel 400. Visual examination of the Monel tubes indicated a multilayered scale and localized attack. The scales were characterized by surface blisters (Figure 3 a) that contained corrosion products and bacteria. The concentration of SRB within these surface deposits was typically 1 x 10^4 cm^{-2}. Removal of the scale exposed localized, irregular-shaped pits that averaged 0.4 mm wide x 1.0 mm deep (Figure 3 b). The EDS spectrum corresponding to unexposed Monel 400 indicated the relative amounts of copper, nickel, iron and manganese in the alloy (Figure 4a). The EDS spectrum from the black layer within the pits showed an accumulation of sulfur and chlorine along with increased amounts of iron compared to the base alloy (Figure 4b). Physical removal of the biofilm from the pit exposed a copper-rich region that was depleted in nickel and iron (Figure 4c).

During the test period, the Gulf of Mexico estuarine water temperature varied from 9°C to 30°C, pH varied from 7.4 to 8.2; and chlorinity varied from 4.3 to 20.0 °/.. Cl^- indicating a range of salinity from 7.7 to 37. Total suspended solids ranged from 10 to 65 mg l^{-1}. Dissolved oxygen varied from 4.3 mg l^{-1} during the summer to 10.8 mgl^{-1} during the winter months. Dissolved sulfides were below detection limits. The mean concentration for sulfates (SO_4^{-2}) was 2.5 gm l^{-1}. The SRB concentration in the estuarine water was 1 x 10^2 to 10^3 cc^{-1}.

Laboratory Testing

Initial Characterizations of SRB From the Mixed Communities. SRB are found in natural water of all salinities from near zero to saturation. Above 2% NaCl the population is almost always *Desulfovibrio*.[22] Most SRB obtained from the marine environment (cultures I-VI in Table 2) have an absolute requirement for 0.5% or greater concentration of NaCl. There were SRB from cultures II and IV that had an absolute requirement for 1.0% or greater concentrations of NaCl. The SRB grew equally well in NaCl concentrations from 1.0 to 4.0%. From culture VII (non-marine environment), pure cultures of SRB were obtained that grew on 0% NaCl but grew equally well on seawater concentrations of NaCl (facultative halophile).

Variations in SRB colonies and cell morphologies from some of the mixed communities suggested that different strains and/or species of lactate-utilizing SRB were isolated. Many of the pure culture SRB isolates from mixed cultures I-VII (marine and non-marine) were desulfoviridin-positive. From this and salt tolerance tests, it appears likely that these pure cultures are *Desulfovibrio*. From the non-marine culture VII, SRB were obtained that showed no fluorescence (desulfoviridin-negative). Further characterization of the SRB (in pure and mixed cultures) will involve the use of fluorescent 16s rRNA probes for SRB. Some of the SRB will be identified using gas chromatography on total fatty acid composition by Microbial ID, Inc. (Newark, DE) or by an in-depth study of the physiology, metabolism, total fatty acid composition and growth optimization by ATCC (Rockville, MD). Table 2 shows resistance/sensitivity of the SRB to five antibiotics commonly used in microbial genetics studies and results for the desulfoviridin test.

All copper-containing metals exposed to anaerobic cultures containing SRB were covered with black sulfur-rich deposits (Figure 5 b,d). Samples exposed to growth medium only exhibited a thin black tenacious layer on 99Cu and no black deposits on brass (Figure 5 a,c). The thickness of the surface deposits on 99Cu in the presence of SRB varied among the cultures (Figures 6 a,b, Table 3). The tenacity of the corrosion products varied with material and culture.

Sulfide layers on 99Cu were consistently nonadherent and in the case of culture VI most of the surface deposits sloughed into the growth medium (Figure 7). Sulfide corrosion products on copper alloys were more adherent. Sulfide layers on all brass samples were thin, tenacious and difficult to scrape from the surface. In all cases, bacteria were closely associated with the sulfur-rich deposits (Figure 8 a-d). Many of the bacteria were encrusted with deposits of copper sulfides (Figure 9). Figure 10 is an EDS spectrum collected from the surface of CDA 706 colonized by culture VII. Surface deposits were enriched in silicon, phosphorus, sulfur, manganese, iron and nickel. Table 4 is a summary of the elemental composition of 90Cu:10Ni and 70Cu.30Ni before and after colonization by cultures II and VII, respectively. In both cases the sulfide layers were enriched in iron and nickel. The biofilm on 90Cu.10Ni contained elevated amounts of manganese. The surface chemistries on brass were not evaluated because the EDS software cannot quantify zinc.

Corrosion under the uniform sulfide layers can best be described as uniform. Localized corrosion was observed under discrete deposits on the surfaces of all alloys (Figure 11).

DISCUSSION

Copper alloys prevent or retard the settlement of macrofouling species such as barnacles and mussels.[23-25] However, bacteria, microalgae, protozoa and their cellular exudates form a slime layer on copper-containing surfaces.[26-28] Marszalek et al.[17] documented that copper fouled at a slower rate than stainless steel and glass surfaces, that fungi were conspicuously absent on copper surfaces and that diatoms appeared only after copper surfaces were covered with a microfouling layer of bacteria/extracellular polymer. Both macrofouling and microfouling increase as the concentration of nickel in the alloy increases.[29]

In the two field studies reported in this paper, SRB were isolated from localized deposits associated with pits. SRB are a diverse group of anaerobic bacteria that can be isolated in many anaerobic habitats, including the marine environment where the concentration of sulfate in seawater is high (typically 2 gm l^{-1}) and fairly constant.[30] Seawater piping systems have high surface areas at which nutrients can concentrate, predisposing these systems to biofilm formation.[31] The metal/biofilm interface can become anaerobic and provide a niche for sulfide production by SRB.[32]

Little et al.[33,34] published several reports documenting localized corrosion of copper alloys by SRB in estuarine environments. Others reported the failure of copper alloys in polluted seawater containing waterborne sulfides that stimulate pitting and stress corrosion cracking.[35] Copper alloys suffer accelerated corrosion attack in seawater containing 0.01 ppm sulfide after 1-day exposure.[36] Pope[13] isolated SRB from 90Cu.10Ni surfaces under deposits of iron- and manganese-depositing bacteria and under discrete deposits of slime forming organisms where severe pitting was observed. The pits under the deposits contained up to several million SRB per pit. It was proposed that the deposits were formed by the action of slime-forming organisms and iron- and manganese depositing bacteria precipitating metals in the hydroxide forms. These surface deposits provide an anaerobic environment that permits the growth of SRB and the production of corrosive metabolic products.

Corrosion of the Monel 400 tube was characterized by pit formation under blisters containing SRB. Differential aeration cells appear to have been created, with low concentration of oxygen immediately underneath the deposits creating an anaerobic environment for SRB. The surface area under the deposit becomes anodic and corrodes while the electrons that are generated react at the cathodic region of high oxygen concentration. Chlorine and sulfur had accumulated within the pit and reacted with the iron and nickel in the alloy. There was a selective leaching of nickel from the alloy leaving a spongy copper-rich material in the base of the pit.

Monel has a tendency for the initiation of pitting in chloride-containing environments where the passive film can be disturbed. Under stagnant conditions, chlorides can penetrate the passive film at weak points causing pitting attack. Sulfides can cause either a modification of the oxide layer or a breakdown of the nickel oxide film. Pit initiation and propagation depend on the depth of exposure, temperature and presence of surface deposits.[8,9] Gouda et al.[37] demonstrated pitting of Monel 400 tubes exposed in Arabian Gulf seawater. Pits developed under deposits of SRB and nickel had been selectively dealloyed.

Because of the diversity among naturally-occurring SRB, seven mixed cultures of strict and facultative anaerobes were isolated from a variety of corroding materials, maintained in the laboratory, partially characterized and used to inoculate copper-containing metals. The substratum to which the microbes attach plays a major role in biofilm processes and may influence the rate of cell accumulation as well as the distribution of cells. Corrosion and sulfide film formation on copper-containing metals were followed using ESEM/EDS. ESEM offers a non-destructive method for imaging wet biofilms on surfaces taken directly from liquid medium without any sample preparation. Also, EDS analysis can be performed at the same time the sample is being viewed and photographed.

The ability to measure wet corrosion layers on metal surfaces demonstrated that the degree of surface derivatization by microbiologically produced sulfides depends on the alloy composition and the specific consortium of microorganisms. A porous layer of cuprous sulfide with the general stoichiometry Cu_2-xS, O<x<1 forms in the presence of sulfide ions.[38] Copper ions migrate through the layer, react with more sulfide, and produce a thick, black scale (Figure 7b, d). It has been argued that if the copper sulfide layer were djurleite ($Cu_{1.96}S$) the sulfide layer would be protective.[39] In separate experiments,[40] djurelite was identified in the x-ray crystallography patterns on 99Cu and 90Cu.10Ni exposed to cultures II and IV. However, even if such a sulfide film were technically passivating, the film's mechanical stability is so poor that sulfide films are useless for corrosion protection. In the presence of turbulence, the loosely adherent sulfide film is removed, exposing a fresh copper surface to react with the sulfide ions. For these reasons turbulence induced corrosion and sulfide attack of copper alloys cannot easily be decoupled. In the presence of oxygen, the possible corrosion reactions in a copper sulfide system are extremely complex because of the large number of stable copper sulfides,[41] their differing electrical conductivities, and catalytic effects. Syrett[38] reviewed the transformations between sulfides and oxides that result in changes in volume and bond weakening, leading to spalling.

A very important feature of wet biofilms on copper-containing metals was that the microorganisms were distributed throughout the copper/nickel/iron-rich surface layers and not on top of these layers as some traditional scanning electron

micrographs have indicated. The microbiologically produced corrosion layers were enhanced in iron and nickel relative to the base alloy suggesting selective dealloying. Manganese that accumulated within the biofilm (Figure 10) must have been bound from the culture medium. Wagner et al.[42] demonstrated metal binding by a marine bacterium on copper surfaces. Metal-binding is easier to detect on wet, unprepared biofilms. The role of polymer-bound metals in the corrosion of copper alloys has been addressed by Geesey.[16]

CONCLUSIONS

Microbiologically produced sulfides appear to react preferentially with iron and nickel in copper alloys. Any benefits these alloying constituents provide to the corrosion protection of the alloy are lost in the presence of SRB where selective dealloying is suggested.

The lack of tenacity of sulfide films on high copper alloys is a factor in the enhanced corrosion observed in the presence of SRB. Sulfide films slough from the surface, exposing fresh copper to react with the microbiologically produced sulfides. The inability to isolate SRB from localized corrosion on the surface of high copper alloys may be due to turbulence in the system or loss of sulfide layers during sample manipulation.

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Table 1. Summary of mixed microbial cultures originally isolated from corroding naval materials in marine and non-marine environments.

CULTURE	METAL	PRIMER	TOPCOAT
I	4140 Steel	5 Step Iron Phosphate	None
II	4140 Steel	5 Step Iron Phosphate	Epoxy
III	4140 Steel	Zinc	None
IV	4140 Steel	IVD-Aluminum	Nylon
V	4140 Steel	5 Step Iron Phosphate	None
VI	Carbon Steel	Proprietary	Proprietary
VII	Aluminum Alloy	Epoxy	Polyurethane

Table 2 Antibiotic and desulfoviridin screens on pure cultures of marine and non-marine sulfate reducing bacteria.

CULTURE	Amp[a]	Tet[b]	Kan[c]	Str[d]	Chl[a]	Desulfoviridin[f]
I	+	+	-	-	-	+
II	+	+	-	-	-	+
III	+	+	-	-	-	+
IV	+	+	+	+	-	+
V-#1	+	+	+	-	-	+
V-#2	+	+	-	-	-	+
VI	+	-	-	-	-	+
VII[g]						
+NaCl#1	+	+	-	+	-	+
+NaCl#2	+	-	-	-	-	+
-NaCl#1	N.D.[h]	N.D.	-	-	-	-
-NaCl#2	N.D.	N.D.	-	+	-	-

[a] Amp, 50 micrograms ampicillin/ml.

[b] Tet, 15 micrograms tetracycline/ml.

[c] Kan, 50 micrograms kanamycin/ml.

[d] Str, 25 micrograms streptomycin/ml.

[e] Chl, 10 micrograms chloramphenicol/ml.

[f] The desulfoviridin test is described in the materials and methods section of this report. +, desulfoviridin-positive result and is evidence for the isolate being *Desulfovibrio*; -, desulfoviridin-negative isolate.

[g] +NaCl, SRB isolates that require 0.5% or greater concentrations of NaCl but these isolates also grew without any NaCl added to the medium.

[h] N.D., Not determined.

Table 3. Thickness of copper foils and sulfide layers after 4 months incubation.

	Thickness of Base Metal (m)	Thickness of Sulfide Layer (m)
Sterile, Unexposed	128	0
Medium - NaCl	120	6
Medium + NaCl	110	12
Culture 1	100	3-5
Culture 2	80	6
Culture 3	60	8
Culture 4	65	3
Culture 5	80	6
Culture 6	70	4

Table 4.

	90/10 Base Metal	90/10 Colonized With Culture II	90/10 With Biofilm Removed	70/30 Base Metal	70/30 Colonized With Culture VII	70/30 Under Biofilm
Si		0.67			0.69	
P		0.61			0.68	
S		7.44	14.57	0.006	11.06	9.31
Mn	0.5	0.92	0	0.78		
Fe	1.4	25.95	5.48	0.51	15.82	3.83
Ni	9.6	20.06	2.91	29.43	28.78	15.46
Cu	88.2	44.34	77.03	69.09	42.97	71.41
Zn	0.1			.02		

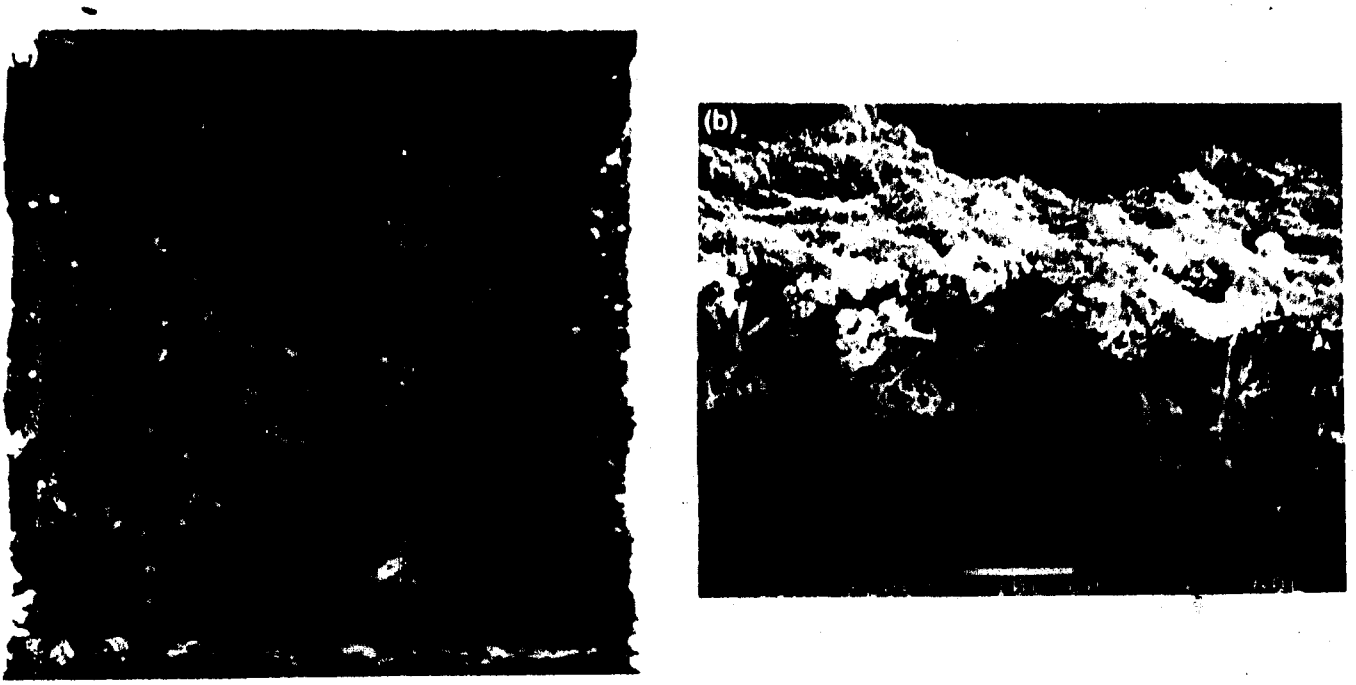

Figure 1. (a) Cross-section of CDA 706 piping after one year in seawater service showing thick surface deposits and pitting; (b) SEM micrograph of bacteria in cross-section of pitted area.

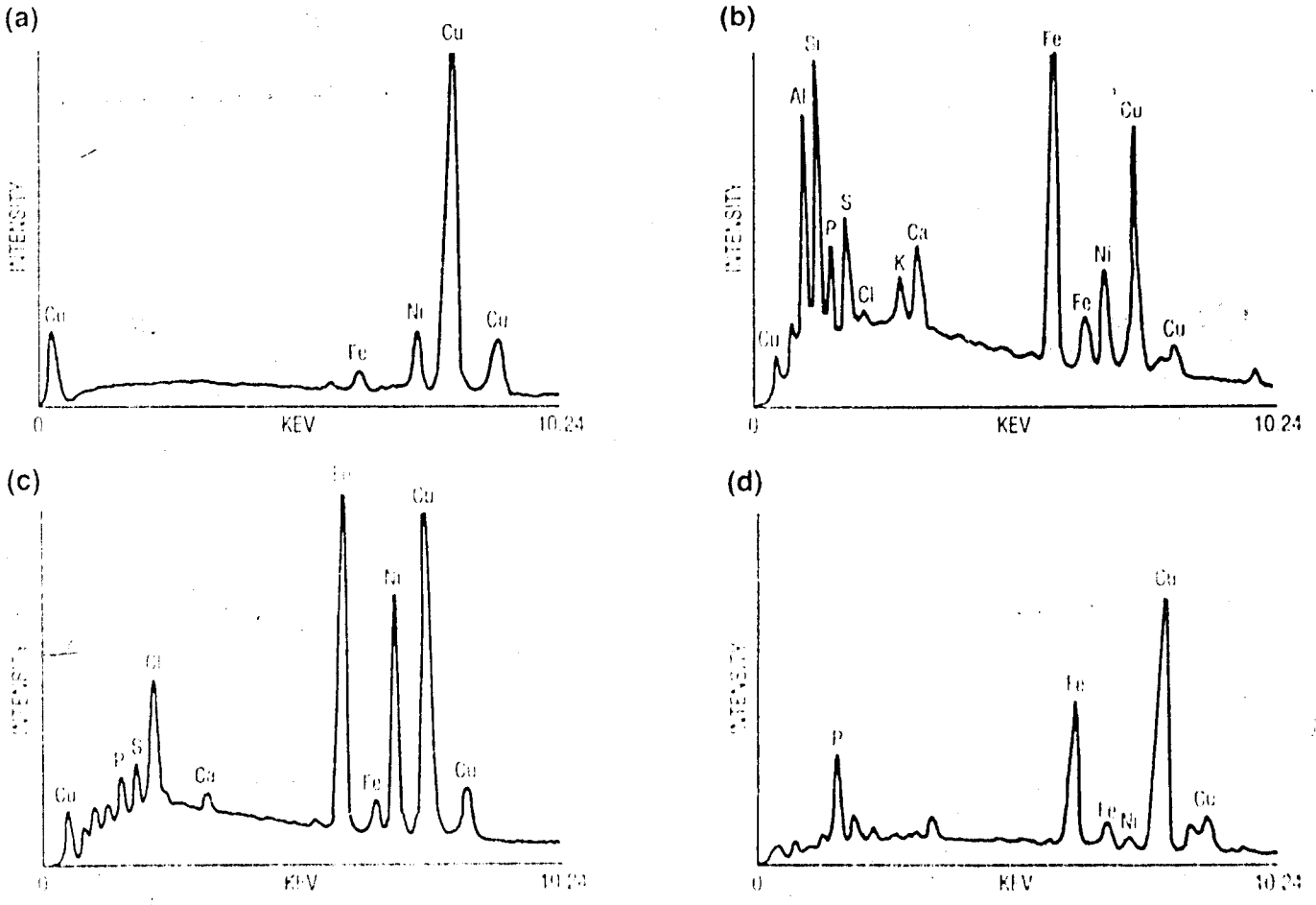

Figure 2. (a) EDS spectrum of clean CDA 706 before exposure; (b) EDS spectrum of pitted region of CDA 706 showing an accumulation of aluminum, silicon, phosphorus, sulfur, calcium and elevated amounts of iron and nickel; (c) EDS spectrum of pitted region of CDA 706 showing the accumulation of chlorine and elevated amounts of nickel; and (d) EDS spectrum of the spongy material beneath bacteria showing an accumulation of phosphorus, an enrichment of iron, and a depletion of nickel in the base of the pit.

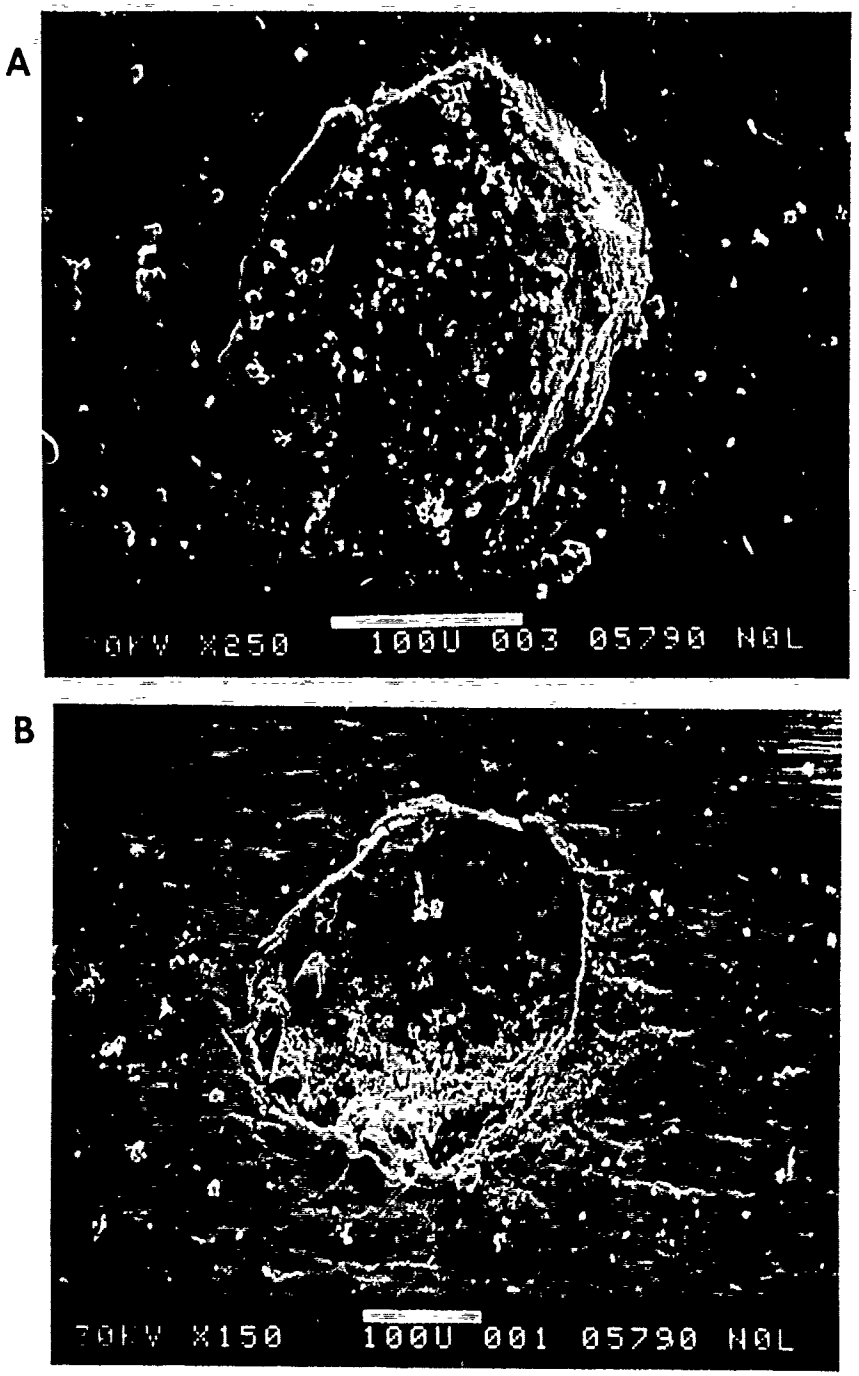

Figure 3. (a) Blister on the surface of Monel 400 tubing after the six-month exposure to estuarine water; (b) Pit in Monel 400 tubing after the six-month exposure to estuarine water.

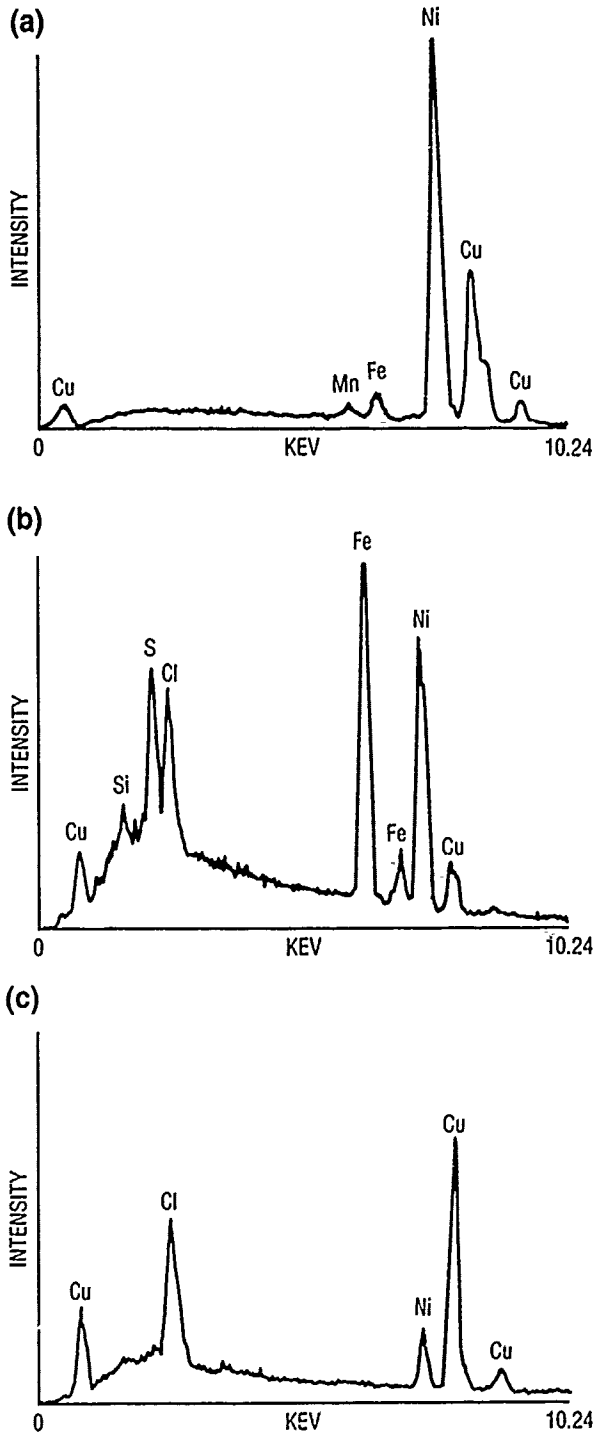

Figure 4. (a) EDS spectrum of unexposed Monel 400, (b) EDS spectrum of Monel 400 after exposure to estuarine water for six months showed an accumulation of silicon, sulfur, and chlorine with elevated concentrations of iron and nickel, (c) EDS spectrum of the residual metal in the base of the pit showing nickel depletion and copper enrichment.

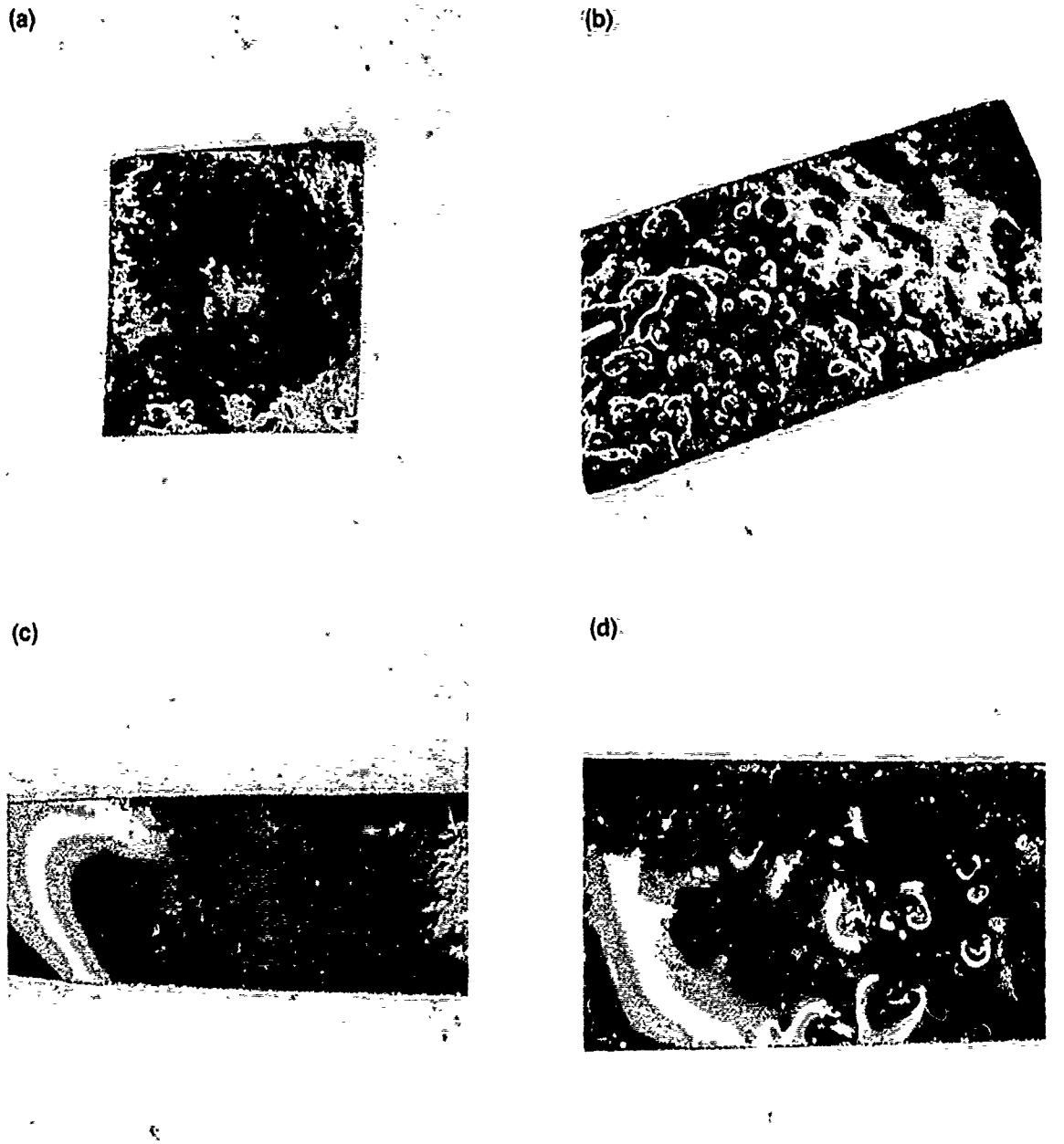

Figure 5. (a) Surface of 99Cu after 4-month exposure to growth medium-NaCl with no SRB added, (b) Surface of 99Cu after a 4-month incubation with culture III, (c) Surface of brass foil after 4-month exposure to growth medium NaCl with no SRB added; (d) Surface of brass foil after 4-month incubation with culture I.

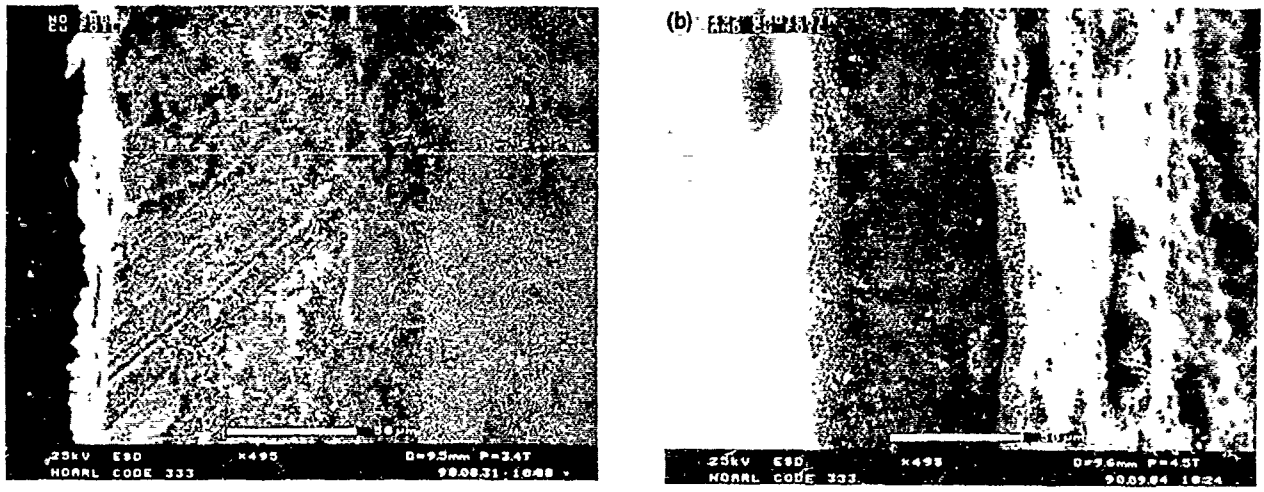

Figure 6. (a) Edge view of 99Cu foil before exposure, and (b) Edge view of 99Cu after 4-month incubation with culture III

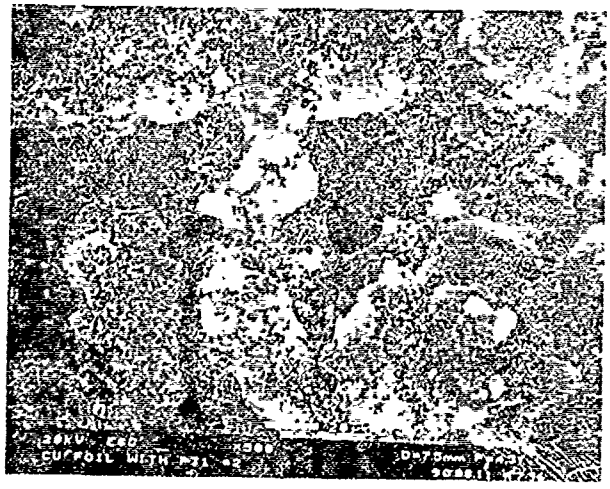

Figure 7 Surface of 99Cu after 4-month incubation with culture VI

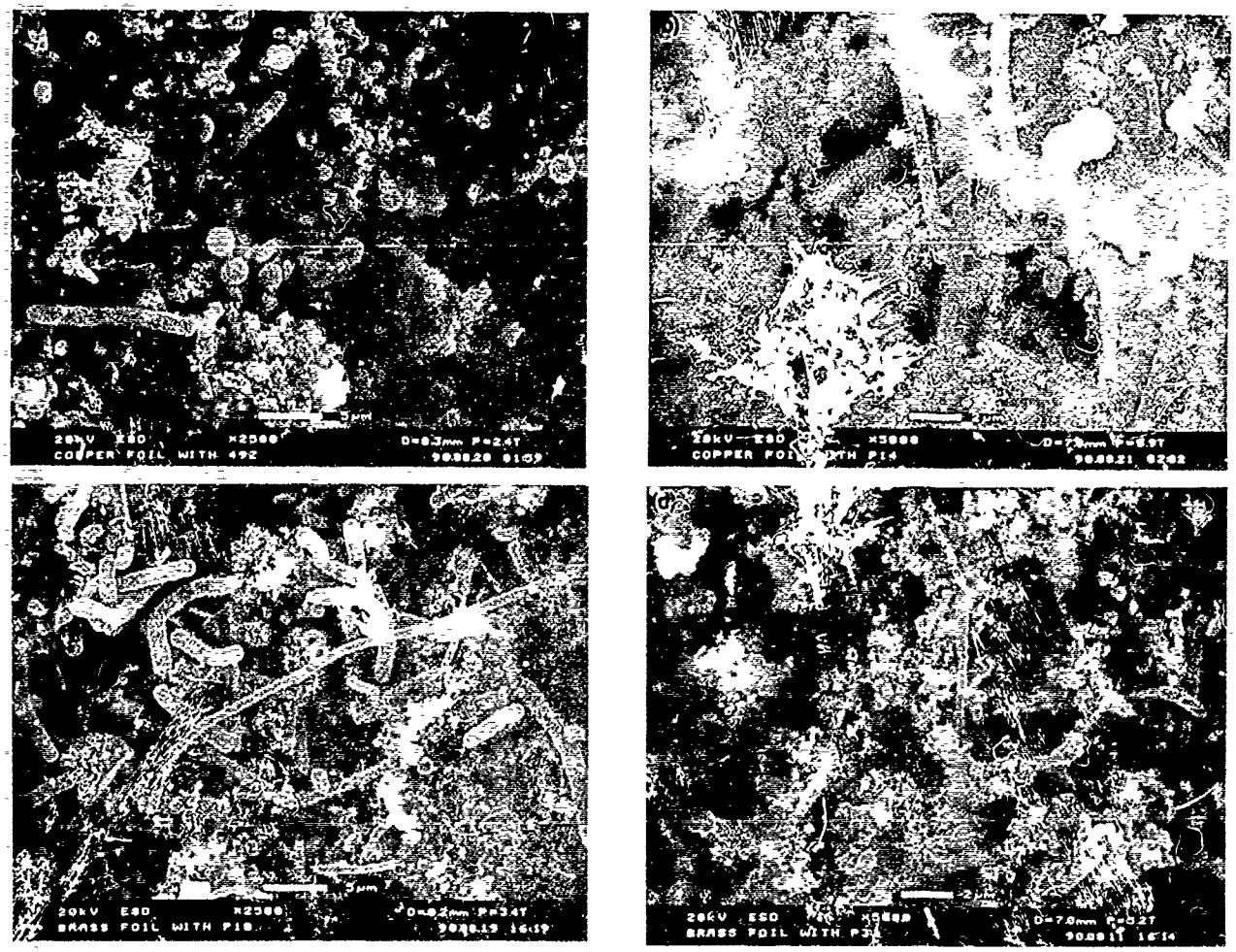

Figure 8. ESEM micrographs of biofilms on: (a) Copper foil after 4-month incubation with culture II, (b) Copper foil after 4-month incubation with culture III, (c) Brass foil after 4-month incubation with culture I, and (d) Brass foil after 4-month incubation with culture V.

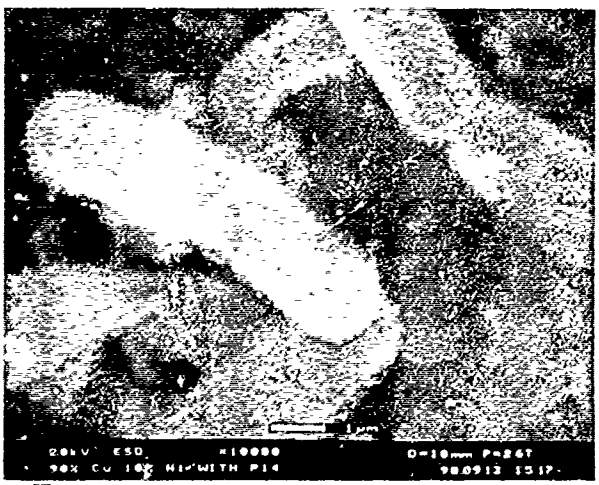

Figure 9. Sulfide deposits on cell from culture II on 90Cu. 10Ni detected by ESEM/EDS.

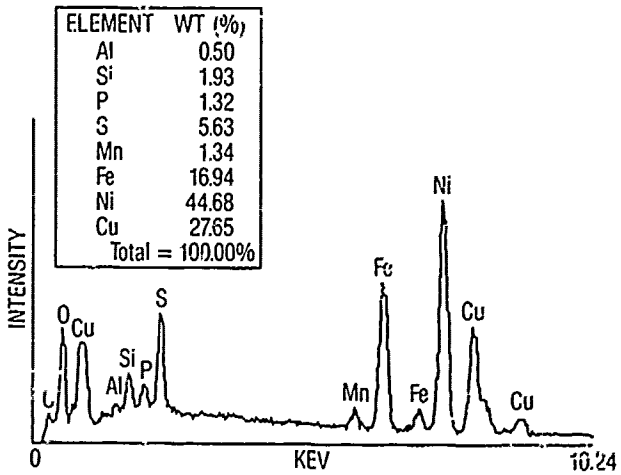

Figure 10. EDS spectrum collected from the surface of CDA 706 colonized by culture II.

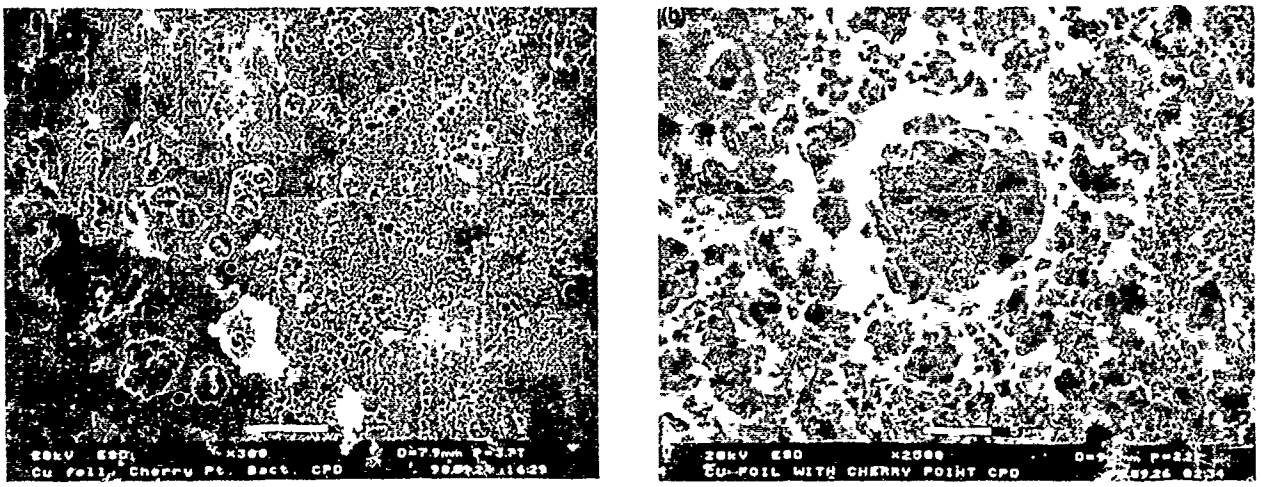

Figure 11 (a, b, Pits on 99Cu foil after 4-month exposure to culture VII